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THE LIFE HISTORY OF POLYSIPHONIA VIOLACEA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXIII.

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THIS preliminary paper will give a brief sketch of my cytological studies on *Polysiphonia violacea* Grev., which were begun last summer at the Marine Biological Laboratory, Woods Hole, Mass., where I occupied a Carnegie Research Table, and were continued at the Hull Botanical Laboratory as a Fellow in the University of Chicago. The problem was suggested by Dr. BRADLEY M. DAVIS, to whom I wish to acknowledge my great indebtedness for his assistance and criticism during the progress of the investigation.

Many points, which for the sake of brevity are omitted in this paper, together with a discussion of literature will be presented in a detailed account with plates to be published later.

METHODS.

The material was killed and fixed in Flemming's fluids, in several modifications which contained the osmic acid in various proportion, Hermann's fluid, 1 per cent. picric acid, and others; among which the weaker formulae of Flemming proved most effective. The best fixation for the study of spermatogenesis and the germination of carpospores and tetraspores was obtained in material killed in weak chrom-acetic acid (Flemming's formula), without any osmic, as follows: 1 per cent. chromic acid, 25^{cc}; 1 per cent. glacial acetic acid, 10^{cc}; sea water, 65^{cc}.

Material was left in the fixing fluid five to forty minutes, and then washed in a gentle stream of sea water. If material remains for a longer time in chrom-acetic acid it becomes very soft and breaks apart. The washed material was passed very gradually through a series of alcohols beginning with 30 per cent., and imbedded in 52° paraffin. The sections were cut 3–5 μ in thickness and stained with safranin-gentian-violet or with iron-alum-haematoxylin, sometimes followed by some plasma stains as orange G, Bordeaux red, Congo red, or safranin. Preparations were studied with a Zeiss apochromatic immersion 1.5^{mm}, N. A. 1.30, and compensating oculars.

GERMINATION OF THE CARPOSPORE AND TETRASPORE.

It is very easy to obtain the early stages in the germination of carpospores and tetraspores. Fruiting plants, placed in a dish of sea water over night, will discharge great quantities of spores. These fall to the bottom of the dish and germinate at once. The germinating spores may be readily gathered from the bottom at the proper hours to obtain critical stages.

The first division of the carpospores and tetraspores takes place within 10–15 hours after their escape from the parent plants.

The cytoplasm before the first division shows a coarse network or very irregular alveolar structure on the periphery, which becomes much finer around the nucleus. The nucleus has a very delicate membrane, within which lies the linin network, much finer in structure than that of the cytoplasm. The delicate transverse walls of the alveoli of the cytoplasm seem to end on the nuclear membrane where the linin thread starts, which leads the writer to believe that there is some relation between the positions of the walls of the cytoplasmic alveoli and the linin of the nuclear network. The nucleus contains one or two nucleoli homogeneous in structure.

Approaching the prophase of mitosis the linin threads become more and more conspicuous and chromatin granules appear in rows; but without constructing a uniform continuous spirem the threads segment into a number of chromosomes. The nucleus becomes surrounded by dense kinoplasm consisting of very minute closely crowded granules, and the outer margin of this kinoplasmic mass assumes a fibrillar structure which finally ends in the alveoli of the cytoplasm. The distinct concentration of the kinoplasmic masses at the poles to become the centers of the dynamic activities of the mitosis does not occur until the chromosomes are arranged in an equatorial plate. The nuclear membrane persists through the prophase, which makes it evident that the spindle is entirely intranuclear in origin.

The chromosomes at the equatorial plate split longitudinally, and the two groups of daughter chromosomes pass to the opposite poles of the spindle, where they become closely crowded in a mass near the center of the accumulation of kinoplasm.

The nucleoli sometimes fragment into two or three small globules, or decrease in size without fragmentation, finally vanishing during the late prophase. New nucleoli appear after the formation of the daughter nuclei. No such close relation seems to exist between the linin thread and the nucleolus as to warrant a belief that in Polysiphonia the nucleolar substance passes directly into the linin thread to form the chromosomes, as is reported in Nemalion.

No centrosomes could be found during this process of mitosis, although the kinoplasm surrounding the nuclear membrane becomes denser during later prophase and finally accumulates at the poles of the spindle at the time of the metaphase. The masses of kinoplasm present no radiations, yet it seems probable that they function as centers of dynamic activity during mitosis, persisting until the daughter nuclei are organized.

The mitoses within the germinating carpospore and tetraspore correspond in all essentials, except that it became at once apparent in the investigation that the nucleus of the carpospore contained about twice as many chromosomes as that of the tetraspore. Counts of the chromosomes made during the later prophase and metaphase of the mitosis made it clear that the nucleus of the carpospore contains about 40 chromosomes and that of the tetraspore 20.

SPERMATOGENESIS.

The mitoses in the vegetative cell of male or antheridial plants will be described before those of spermatogenesis. During the prophase the chromatin granules increase in size and become grouped as a number of short rod-shaped bodies upon the linin thread, without developing a regular and uniform chromatin spirem. The linin thread then segments into 20 chromosomes.

Polysiphonia, as a rule, is dioecious; however, cystocarpic plants sometimes produce antheridia, a condition which will be described later with other abnormalities. The antheridia develop as lateral branches near the tips of the main filaments. Each branch consists of a central axis from which clusters of sperm mother-cells or spermatocysts arise at the side on short stalk cells.

The cytoplasm of the spermatocyst has a delicate granular structure and contains a large vacuole, generally in the center of the cell. Its nucleus in the resting state is similar to that of the vegetative cell, and during prophase the linin network becomes more conspicuous and finally segments into 20 chromosomes. The kinoplasm around this nucleus is rather scanty, even after the spindle fibers are formed. The spindles are intranuclear. No centrosome could be found, but there is a concentration of kinoplasm at the two poles.

After anaphase, the nuclear membrane dissolves and the vacuole intrudes into the nuclear cavity between the two sets of daughter chromosomes, one set passing to the apex and the other remaining at the base of the sperm mother-cell. The upper part of the cell, including the vacuole, is then cut off as the sperm from the lower portion by a cleavage furrow, which crosses the cell somewhat obliquely.

The nucleus which remains at the bottom of the sperm mother-cell now repeats this mitosis, forming a second sperm, and perhaps two or three more are developed before the antheridium ends its fertility.

OOGENESIS AND FERTILIZATION.

The mitoses in the vegetative cells of the female or cystocarpic plant are similar to those of the male. The number of chromosomes is invariably 20.

The female organ or procarp develops from a central axial cell, next to the apical cell of a short lateral branch. The central axial cell gives rise to a pericentral cell from which by successive mitoses a four-celled carpogonial branch is formed. The terminal cell of this series becomes the carpogonium, situated as a rule above the pericentral cell, owing to the curved growth of the carpogonial branch.

The carpogonium is at first round and the nucleus lies in its center. While this nucleus undergoes a typical mitosis to form two nuclei, the carpogonium puts forth the process which is to become the trichogyne. After mitosis one of the nuclei proceeds into the developing trichogyne to become a trichogyne nucleus, and the other remains below in the carpogonium as the gamete nucleus.

Coincident with the development of the trichogyne, the pericentral cell gives rise to the two sets of auxiliary cells, one of which is a series of three or four, formed above, so that they lie just beneath the carpogonium, and the other series consists of two cells formed below.

When the sperm comes in contact with the trichogyne, the walls between dissolve, the contents of the sperm cell enter the cytoplasm of the trichogyne, and the sperm nucleus passes down into the carpogonium where the fusion of the male and female nuclei takes place.

The trichogyne nucleus may be found even after the sperm nucleus has passed into the carpogonium. But later, when the trichogyne becomes separated from the carpogonium, its nucleus can scarcely be distinguished. The trichogyne then shrivels and dies.

The carpogonium after fertilization unites with one of the auxiliary branch cells which lies beneath, and the latter also fuses with the pericentral cell, thus providing a passage into the pericentral cell for the fusion nucleus of the fertilized carpogonium. Then the remainder of the auxiliary branch cells fuse with one another and with the pericentral cell, which results in a large fusion cell, the central cell, that naturally contains a number of nuclei.

The nuclei in the central cell are of two sorts with respect to origin: first, there is the fusion nucleus from the carpogonium (sporophytic); and second, there are a number of nuclei derived from the auxiliary cells, which are of course gametophytic. The fusion nucleus gives rise to a series of nuclei by typical mitoses which present 40 chromosomes as a sporophyte number. The central cell now develops several lobes into which these sporophytic nuclei pass. Further mitoses increase this number, and each lobe then cuts off a carpospore terminally, which is attached to the central cell by a short stalk.

After the carpospores are formed, the central cell increases in size greatly, absorbing the stalk cells, and even the central axial cell also becomes involved in this general cell fusion. These very extensive cell unions are probably concerned with the nourishment of the carpospores.

Some of the gametophytic nuclei derived from the auxiliary cells remain in the central cell, increasing in size and finally breaking down after a number of peculiar changes. Others of the gametophytic nuclei divide amitotically to form the paranematal filaments which lie under the wall of the cystocarp.

TETRASPORE FORMATION.

The tetrasporic plant normally never produces antheridia or procarps, and the cytological studies on the vegetative cells give proof that it differs in an important respect from the sexual plants. The mitoses in growing regions of the tetrasporic plant show that the nuclei have 40 chromosomes (the sporophyte number), while it will be remembered that the nuclei of the sexual plants have 20.

I shall not enter at this time into a detailed description of the events which take place during the formation of the tetraspore mother-cell; the only thing to be remembered is that the number of the chromosomes appearing during this mitosis is 40, so that it follows that the nucleus of the tetraspore mother-cell contains 40 chromosomes.

The nucleus of the tetraspore mother-cell increases somewhat in size, accompanied by the growth of the cell itself; yet the latter is relatively slow until just before the first mitosis of the nucleus, but very rapid after that.

The resting nucleus of the tetraspore mother-cell contains a fine network of linin in which the chromatin is distributed irregularly in larger and smaller granules. The nucleolus has no visible connection with the linin thread. With the further growth of the nucleus the linin thread increases in thickness; in such an irregular way, however, that in some parts the threads are uniform in thickness and in the others they appear to have knots. The chromatin thread now forms a fairly well-developed spirem.

This condition presently passes into the so-called stage of synapsis, when the spirem consists visibly of two parallel threads close together, while in the other parts the two are in contact side by side or fused into a single thread. The two threads may represent, according to recent interpretations of synapsis, chromatin of maternal and paternal origin.

After synapsis, the tangled thread becomes distributed throughout the cavity of the nucleus. The spirem now shows the longitudinal fission which precedes the separation of chromatin granules into two sets, and then the spirem segments into 20 chromosomes, each showing clearly its bivalent nature.

While this process of chromosome formation is going on in the interior of the nucleus, the kinoplasmic material surrounding the nucleus becomes concentrated at two poles of a spindle, and when the chromosomes are arranged in the equatorial plate a minute body occupies the center of each pole. The body might be called a centrosome, but it has not been possible to recognize its presence during prophase or to follow it after anaphase.

It is interesting to note that the two poles are not in a straight line perpendicular to the center of the equatorial plate, but are asymmetrical and less than 180° apart. Each of the 40 sporophytic chromosomes composing the 20 pairs (bivalent chromosomes) arranged in the equatorial plate now splits longitudinally, so that a large number of univalent chromosomes results, probably 80 in all, although I was unable to count the exact number.

The group of 80 grand-daughter chromosomes separates into two sets, but resting neuclei are not formed, and their further distribution by the second mitosis begins at once. The axes of the two spindles of the second mitosis lie perpendicular to each other, and their complex relation to that of the first will be described in the final paper. Kinoplasmic masses occupy the poles of the spindles in the second mitosis, and each contains a centrosome-like granule.

Now, each group of 40 chromosomes, following this first mitosis, separates into two sets of grand-daughter chromosomes, 20 in each set, which are attracted toward the respective poles of the two spindles, where the kinoplasmic material becomes more pronounced than before. These chromosomes, after reaching the four poles, become massed together, lose their individual outlines, and larger and smaller granules appear shortly after on linin threads which later become contained in the four daughter nulcei.

It is a remarkable fact that the membrane of the original nucleus in the tetraspore mother-cell persists through the two mitoses which have just been described. The area included by this membrane increases in size with the growth of the cell. The cytoplasm around it shows larger alveoli, which become smaller in the vicinity of the original nuclear membrane, and at last passes into the kinoplasmic fibrils immediately surrounding it.

At this time constrictions appear simultaneously in the area marked by the original nuclear membrane. The kinoplasm intrudes into this area in a very interesting manner, which cannot easily be described without figures, but results in the organization of the four daughter nuclei that are to be contained in the tetraspores.

The division of the tetraspore mother-cell does not take place simultaneously with the events described above. Cleavage furrows start along four lines on the periphery of the cell, a little before the end of the nuclear division, and proceed more rapidly after its completion. During the entire process of the tetraspore formation, the mother-cell is connected by a strand of protoplasm with the stalk-cell, and probably gets much nourishment through this strand, for the tetraspore mother-cell increases greatly in size. Finally, the cleavage furrows meet in the center between the four daughter nuclei, thus dividing the protoplasm into four tetraspores.

ABNORMALITIES.

Normally, the male and female organs and the tetraspore are never developed in the same plant, but it often happens that the male and female organs are produced on the same individual, and occasionally antheridia are formed on the same branch with procarps and cystocarps. The sexual cells in these cases are developed normally, the number of the chromosomes being always 20. Cystocarpic plants have also been found producing cells whose lineage is identical with that of the tetraspore mother-cell. However, I have never seen evidence of nuclear division in such a cell; cleavage furrows appear and cut deeply into the protoplasm, which nevertheless remains undivided, so that tetraspores are not formed. Whether this cell may escape and germinate as a monospore has not yet been determined.

CONCLUSION.

The nuclear conditions in the life history of Polysiphonia may be summarised as follows:

1. The germinating carpospore contains 40 chromosomes, and the tetrasporic plant the same number; so it may be inferred that the tetrasporic plants come from carpospores.

2. The germinating tetraspore contains 20 chromosomes, and the sexual plants (gametophytes) the same number; so it may be inferred that the sexual plants come from tetraspores.

3. The nuclei of the gametes (sperm and carpogonium) contain each 20 chromosomes. The fusion nucleus (sporophytic) in the fertilized carpogonium presents 40 chromosomes, and gives rise to a series of nuclei. Some of these enter the carpospores, which are consequently a part of the sporophytic phase to be continued in the tetrasporic plant. The gametophytic nuclei in the central cell of the cystocarp (with 20 chromosomes) either break down or form the paranematal filaments.

4. Tetraspore formation terminates the sporophytic phase with typical reduction phenomena, so that the tetraspores are prepared to develop the gametophytic generation.

5. There is thus an alternation of sexual plants (gametophytes) with tetrasporic plants (sporophytes) in the life history of Polysiphonia, and the cystocarp forms a part of the sporophytic phase.

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